SYNTHESIS, CHARACTERIZATION, ANTIMICROBIAL AND TOXICOLOGICAL EFFECT OF METAL OXIDES NANOPARTICLES

SYNOPSIS

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1. Title: SYNTHESIS, CHARACTERIZATION, ANTIMICROBIAL AND TOXICOLOGICAL EFFECT OF METAL OXIDES NANOPARTICLES

2. Introduction

The nano field of science found its way in 21st century with the concept of ultrafine particles having size range less than 100nm (Donaldson et al. 2001). Firstly the applications of nanoparticles were applied in medical field, then after knowing its anti-microbial potential, it found application in food industry (Chaturvedi and Dave 2020). Human health is continuously facing micronutrient deficient environment, as per survey, 57% of women are deficient in zinc and 33-41% are iron deficient in early reproductive phase of life (Ara et al. 2019; Vannella et al. 2008). Zinc and iron are highly present in soil, but due to its immobile character, root shoot barrier cause its deficiency in plants. This deficiency in plant is linked directly to malnutrition of microelements in horticulture/agriculture crops resulting in micronutrient deficiency in humans (Sharma et al. 2013). The nano- supplementation in post harvest coatings provides a big way to eliminate the micro-elemental deficiency during *in vivo* plant growth (Sharifan et al. 2021). Metal oxide nanoparticles (MONPs) are the non-toxic inorganic compound taken as supplement and possess antimicrobial activity which leads it entry towards food industry for serving as packaging film to minimize food spoilage by microbial contaimination (La et al. 2021). The ZnONPs is promising tool for post storage because of its antimicrobial activity (Gudkov et al. 2021). The ZnONPs shows anti-bacterial properties against *Bacillus subtilis, Klebsiella pneumonia*, and *Staphyloccocus aureus* (Thakur et al. 2021). ZnONPs also act upon post harvest fungus, *Botrytis cinerea* (Issam et al. 2021), *Cercospora* sp. (Patino-Portela et al. 2021). The chitosan embedded iron oxides nanoparticles were analysed for anti microbial activity against pathogens named *Escherichia coli, Staphyloccocus aureus, Candida albicans* (Konwar et al. 2016). Fe_3O_4 possess better bactericidal property (Sangeetha and Philip 2013). The National Institute of health and food recommended 40mg/day consumption of zinc (https://ods.od.nih.gov/factsheets/Zinc-HealthProfessional/) and 30mg/day consumption of iron (https://ods.od.nih.gov/factsheets/iron-HealthProfessional/) (Heydari-Majd et al. 2019;

Karakoc et al. 2021). In this era humans are more desiring towards fibre based diet by which they are consuming more fruits and vegetables. The protection of postharvest is going on by using chemical fungicides. These chemical fungicides not only spoil food but also hazardous to human health and environment. Thus the nanofield is providing us an alternative of chemical fungicides which can supplement their lifestyle by micronutrient (González-Estrada et al. 2021). MONPs are used as an alternative source of replacing the chemical fungicides as it enhancing the shelf-life of horticultural crops (Luksiene et al. 2020). The human health is impacted by the quality of fruits and vegetables consumed by them. As per concern to the modern life style the demand of processed food is gaining attention (Yousuf et al. 2018). The protection of food against foodborne diseases necessitated the development of antimicrobial food packaging. The combination of inorganic and organic material packaging has proved to be highly efficient in their functionality. The metal oxide NPs provides antimicrobial activities and edible polymers although provides mechanical strength for active packaging (Carbone et al. 2016). The demand of the consumers in food industry is influenced by the quality status of food. The poor storing condition leads to deleterious effects on stored fruits/vegetables and also less accepted by the consumers. Thus sustainable packaging is necessary to deal with the demand of time. The packing must have to fulfil some criteria regarding to extended barrier properties, controlling microbial count, shelf life enhancement, and other physicochemical properties (Mohebbi et al. 2014). Iron oxide nanomaterial possesses efficient food packaging activity which is more suitable in food industries (Thakur et al. 2020).

The nanotechnology found its way in leading attention in engineering, food, and medical research fields. ZnONPs were studied as biocompatible towards normal mammalian cells (Medina-Ramírez et al. 2020). The NPs possess biocompatibility towards normal cells besides it; they also exhibited cytotoxicity against cancer cell line (Haghighat et al. 2021). The ZnONPs of the size range 9±4 shows cytotoxicity and causes enhancement in the activity of pro apoptotic gene while it act antagonistically towards anti-apoptotic genes (Shivyari et al. 2021). The toxicity effect of NPs towards cancer cells were both dose and time dependent (Mohammad et al. 2021). The toxicity evaluation of supermagnetic iron oxide nanoparticles increases its biocompatibility towards normal cell lines (Ghasemi et al. 2018). Our ecosystem and health is continuously harassed by using non-biodegradable, and non-renewable used food packaging materials. So the new idea of green economy is gaining attention in every field of life. Smart nano-packaging film is the newly growing idea which provides sustainability for environment. Nano-science provides nanodevices, nanomaterials, and nanosensors used for monitoring all aspects of packaged food and also friendly to environment (Bumbudsanpharoke and Ko 2019). The fruits and vegetables consumption occur in both fresh and processed form. As per the changing lifestyle the desire of packaged

fresh fruits/vegetables cuttings also increase, which meets the challenge for sustaining the freshness of food by industries (Yousuf et al. 2018). In the proposed research we will be synthesizing metal oxide nanoparticles (zinc oxide and iron oxide nanoparticles) using chemical free, ecofriendly hydrothermal method. Synthesized MONPs will be studied against the opportunistic bacteria and fungi. The biocompatibility evaluation of MONPs will be performed on HEK293 cell line. The work would be carried out by undertaking following objectives:

1. Synthesis and characterization of metal oxide nanoparticles using hydrothermal method.
2. Antimycotic and antibacterial study of synthesized metal oxide nanoparticles against opportunistic pathogenic fungi and bacteria.
3. Storage study of perishable vegetables with metal oxide nanoparticles.
4. Toxicity evaluation of synthesized metal oxide nanoparticles against HEK293 cell line.

3. Expected outcomes of Research

The unique characteristics of metal oxide nanoparticles are useful in food industry. It includes antimicrobial, chemical reduction, ligand sequestration, and thermal stability. Metal oxide nanoparticles are considered to be safe for human as they are used in various metabolic pathways *in vivo* and deals with micronutrient deficiency inside body. Thus they enhance post harvest life without affecting the native form of vegetables and fruits. By producing ecofriendly MONPs, we can enhance shelf life of perishable vegetables and fruits without adding preservatives which will be useful for sustainable environment. Taking into the account of health benefits and storage problems our proposed work will be developed for extending storage life of perishable vegetables by retarding the growth of undesirable microorganism during the storage time. Toxicity evaluation on HEK293 cell line counts for safe use of synthesized metal oxide nanoparticles.

4. Review of literature

Hydrothermal synthesis of nanoparticles is based on controlled temperature and pressure condition in autoclauve. The minimum change in the temperature and pressure can lead to variation in morphological features (Adschiri et al. 2000). Hydrothermal is eco-friendly technique which provides us wide variety structure of nanomaterials by regulating pH, precursor, temperature and time. The structure includes zero dimensional (0D) spherical granular, one dimensional (1D) nanorods, two dimensional (2D) nanoplatelets, and three dimensional (3D) flowers like multibranched structure. The microbicidal property of flower shaped nanostructure was effective (Chang et al. 2020). The impact of variation in temperature and pH influence the structure of NPs which can be characterized by XRD, FTIR, TEM (Wasly et al. 2018). The hydrothermally synthesized flower shaped ZnONPs

structure used to sense gas (Agarwal et al. 2019). The hydrothermal synthesis of ZnO at varying temperature (120 ºC, 150 ºC, and 180ºC) evaluated as wurtzite form of hexagonal structure. The synthesized ZnO at 120 ºC was reported best among others for having better capability for degrading dyes (Júnior et al. 2017). The hydrothermally synthesized and characterized ZnONPs were reported at different temperature (100 ºC- 46nm, 125 ºC- 41nm, 150 ºC- 35nm for 2hr) and time period (120 ºC for 1hr- 45nm, 3hr-54nm, 5hr- 78nm). The variation in temperature/time also effect the structure; at 100 ºC, 125 ºC having 3D flower like structure, 150 ºC having (0D) spherical shape, and 120 ºC for 1hr, 3hr, 5hr showed (1D) nanorod structure (Mohan et al. 2020). La et al. (2021) reported the hydrothermal synthesized ZnONPs at 150 ºC of size range 5-40nm with good separation. Bharti and Bharati (2017) reported the hydrothermally synthesized granular ZnO of size 15.81nm at 180 ºC for 18 hr. The porous ZnO and ZnO@ZnSNPs were synthesized by low temperature approach at 160 ºC for 6 hr. The characterisation showed that the mesoporous ZnO had size of 50 nm with 5nm pores diameter and ZnO/ZnS had 60 nm of size with 15nm of hollow pores (Li et al. 2021). The hydrothermally synthesized ZnO at 160 ºC, for 1 hr of incubation form nano flakes, by extending time for 2 hr it recorded roughly formed hexagonal cylindrical structures, further extension in time gave smooth microcrystals and by extending the time 10 times i.e. for 10 hr, hexagonal cylindrical twinning structure were obtained (Zhang et al. 2021). The TiO_2/ZnO oxides formed by hydrothermal approach having higher surface w.r.t. normal oxide and reported for its photocatalytic and bactericidal activity (Siwińska-Stefańska et al. 2019). CuO@ZnO were hydrothermally synthesized and reported for its efficient phocatalytic properties and long term stability (Prabhu et al. 2019). The Fe_3O_4 of size range 15-31nm were synthesized hydrothermally by maintaining temperature (134ºC) and pressure (2 bar) for 3 hr (Ge et al. 2009). Hydrothermally synthesized iron oxides NPs were studied at the different ranges of time duration. As 180 ºC@12hr the size ranges from 14-30nm, while increased in time duration for 48hr the size of nanoparticles were 21-124nm. They concluded that the time period of reaction process is playing important role in maintaining the size difference in high temperature synthesized iron oxides. The ferromagnetic character of iron oxide nanoparticles were studied and observed that in high size NPs, cause increase in saturation of magnetization (Ozel et al. 2015).

As we are going toward vast production and uncontrolled extensive use of antibiotics, the antibiotic resistance in microbes is the main concern to save human life from life threatening infectious diseases. The novel antimicrobial agent developed by nano-science is the need for these resistant microorganisms. The inorganic nano compounds have been shown to possess potent antimicrobial properties. The antimicrobial property of ZnONPs against *Streptococcus parauberis* showed a clear inhibition zone (Fadl et al. 2021). The green synthesized ZnONPs had bactericidal properties towards gram positive and negative bacteria;

Staphylococcus epidermidis, *S. aureus* and *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli* respectively (Kahsay 2021). The synthesized ZnONPs of 10nm having good surface and volume ratio, showed better antimicrobial properties towards bacteria (*Staphylococcus aureus*, *S. pneumoniea*, *Klebsiella pneumonia*, *Escherichia coli*) and yeast (*Candida albicans*) (Umavathi et al. 2021). The remarkable antipathogenic effect of ZnO against food borne microbes were reported by Busi et al. (2016) against *S. aureus*, *Salmonella enterica*, *Vibrio parahaemolyticus*, and *E.coli*. The ZnONPs (50nm) reported its fungicidal activity against post-harvested fungus isolates of *Alternaria alternata*, *Botrytis cinerea*, *Penicillium expansum*, *Rhizopus stolonifer* and characterisation of damaged inflammated cell was done by SEM imaging. Thus it is good approach for post harvest storage (Sardella et al. 2017). Nowadays traditional oils itself not that much sufficiently shows antimicrobial properties, thereby supplementated with ZnONPs for betterment in its properties (Hassan et al. 2020). Ali et al. (2021) reported the spherical shaped 20-35nm Fe_2O_3NPs potent for inhibition of *Fusarium oxysporum* that causes brown rot in citrus fruits. The green synthesized Fe_2O_3NPs from piper betel leaf extract having size 25nm were found to have bactericidal properties against gram positives (*Staphylococcus aureus*, *Streptococcus*) and gram negatives (*Escherichia coli*, *Pseudomonas aeroginosa*) (Yoonus et al. 2021). (Jelić et al. 2020) studied the anti-microbial activity of copper doped iron oxide NPs against *Staphylococcus aureus*, and *Acinetobacter baumannii.*

The food industries are regularly dealing with postharvest spoilage due to microbial contamination. These losses due to microbes are started very beginning from field, cold stores and then to consumers. The incorporation of silver NPs in low cost biodegradable cellulose packets showed killing effect against *Aeromonas hydrophila* without losing nutritional value of packaged vegetables (Singh and Sahareen 2017). La et al. (2021) reported the freshness of banana for post 17d of storage after coating it with ZnONPs having nontoxic inorganic nature with edible chitosan and arabic gum. The ZnO possess antibacterial activity against both the gram positive and negative bacteria and also maintained its quality. The ZnO synthesized hydrothermally incorporated with chitosan/gum arabic were used as edible coating for maintaining the physiochemical properties of harvested avocados (Le et al. 2021). The ZnONPs are emerging toward enhancing shelf life and micronutrient content in tomato. It reduces the post harvest losses, maintained tomato quality; prevent the microbial contamination, while increasing both lycopene and Zn content in fruit (Sharifan et al. 2021). The ZnNPs were used as supplement in Japanese quails diet. The research showed the betterment in their quality and performance (Reda et al. 2021). The reported ZnO synthesized by green approach and a comparable study was conducted between synthesized and commercial ZnONPs. The post storage study on strawberries showed that both the NPs resulted in antimicrobial activity against microorganism, act antagonistically towards weight

loss and enhanced the storage span (Gao et al. 2020). Malek (2020) reported the storage study of mango by using ZnONPs conducted at 32, 27, and 5 ºC temperature and evaluated that the coated mango stored at low temperature possess delayed ripening, firmness maintained, and minor weight loss. The homogenous dispersion of NPs on mango was characterized by EDX and FESEM. Guo et al. (2020) conducted the storage evaluation of perishable tomato by using hydrothermally synthesized flower shaped ZnONPs supplemented with cinnamaldehyde in carboxymethylcellulose based nanopolymer having less transparency with more flexibility and mechanical stress resistor. This nanocomposite enhances the storage span by acting against both, foodborne fungus and weight loss property. The reinforcement of ZnONPs in xanthan used as edible coating for apple and tomato provided them great bactericidal activity. The edible coating resist weight loss in stored samples, additionally they are biocompatible (Joshy et al. 2020). The ZnO/CuO along with alginate provides better mechanical properties toward activeness in food packaging nanocomposite (Aristizabal-Gil et al. 2019). The carboxymethyl-cellulose-chitosan polymer was studied for storage of bread by introducing ZnONPs (Noshirvani et al. 2017). The CuO/ZnO embedded with chitosan forming nano polymer based composite by solvent casting method. This nanocomposite possesses anti properties against microbes and oxidants due to incorporation of green synthesized nanoparticles, and reported to enrich the shelf life guava (Kalia et al. 2021). The chitosan loaded Fe_2O_3NPs spherical rod shaped NPs of size range 20-30nm were potent antifungal against *Rhizopus stolonifer* that causes soft rot in peach (Saqib et al. 2020). Fe_2O_3NPs were evaluated for post storage of peanuts as it acts as antimycotic against *Aspergillus flavus* (Sun et al. 2021). The calcium oxide NPs were studied for its antimicrobial property for enhancing the storage span of cucumber without showing negative impact on antioxidants, physical appearance, and pigments (Cid-López et al. 2021). The chilling stress on persimmon was decreased by chitosan having phenylalanine NPs. The results concluded that the dip coated fruit showed higher level of antioxidant enzyme to resist this stress (Nasr et al. 2021). Packaging material for managing post-harvest storage of vegetables/fruit crops is still rare in the market. TiO_2NPs incorporated with chitosan reported to control the storage loses in tomato stored at 20ºC temperature (Kaewklin et al. 2018).

The study of nanoparticles/cell membrane interaction based upon confocal fluorescence imaging and dye based fluorimetry techniques have been carried out. The MO and membrane of phospholipids (phosphotidyliositol, phosphotidylcholine) head group specifically interact with each other. The ZnO passively penetrate by phase separated region due to electrostatic force (Tiwari et al. 2018). The toxicity of AgNPs towards HEK293 concluded no toxic effect of AgNPs up to dosage of 120µg/ml (Naveen et al. 2021). Patra et al. (2020) reported the toxicity effect of AuNPs against HeLa while antagonistically biocompatible with HEK293 cell line. Palladium with reduced graphene oxide/Zinc oxide

evaluated as good biocompatibility towards HEK293 cell line (Rajeswari and Prabu 2020). Sangeetha et al. (2021) studied the carbon nanoparticles toxicity on HEK293 cell line and observed that the toxicity effect is dose dependent. The safe range up to which the biocompatibility occurred was 50-200 µg/ml, beyond this range pro apoptotic gene (Bax) increased with decreased in anti-apoptotic gene (Bcl-2) expression. The ZnO@K doped NPs were studied for cytotoxicity and reported it's biocompatible towards normal cell and cytotoxic towards cancer cell lines (Haghighat et al. 2021). The research was conducted to get the toxicity evaluation of ZnO/TiO_2/Ag nanomaterial. The 190µg/ml dosage of this nanocomposite was considered as biocompatible to macrophages moreover the same dose causes cytotoxicity in cancer cells (Sakthi Mohan et al. 2021). The 5-19nm range iron oxide nanoparticles found biocompatible towards normal (HEK293) cell line on low dosage 100µg/ml (Ghasemi et al. 2018). The anti-mitotic activity showed by Fe_2O_4@Mg against cancer cells up to concentration range 43 µg/ml besides it concluded its biocompatibility towards HEK293 up to the range 158 µg/ml (Kardan et al. 2021).

5. Technical programme

5.1 Experiment 1: Synthesis and characterization of metal oxide nanoparticles using hydrothermal method.

5.1.1 Location: Eternal University - Synthesis, CSIR IHBT Palampur and Punjab University – characterization.

5.1.2 Methodology:

- Synthesis of ZnONPs and Fe_2O_3NPs by using low temperature hydrothermal method.
- Preparation of ZnONPs and Fe_2O_3NPs under two different temperature variation (110 ºC, and 150 ºC) with constant pH and time.
- Characterization of synthesized NPs carried out by XRD, RAMAN, FTIR, EDS, SEM, and TEM.

5.1.3 Observations to be recorded: The crystalline structure of synthesized MONPs performed by XRD, RAMAN, EDS; bonding pattern of different groups confirm by FTIR, and the structural confirmation will be done by SEM and TEM.

5.1.4 Statistical analysis: The analysis regarding to proposed work and graph plotting will be performed by using *Origin* software. The software programs such as *JCPDS* will be used to compare the XRD data.

5.2 Experiment 2: Antimycotic and antibacterial study of synthesized metal oxide nanoparticles against opportunistic pathogenic fungi and bacteria.

5.2.1 Location: Eternal University

5.2.2 Methodology:

- Isolation of bacteria and fungi (which causes post harvest losses) from perishable crops capsicum and tomato; Machher local farmer field.

Isolates / Crop	Bacterial	Fungal
Capsicum	*Staphylococcus aureus*	*Fusarium incarnatum*
Tomato	*Klebsiella pneumonia*	*Aspergillus niger*

- Morphological studies, DNA isolation, identification and sequence submission of the isolates in NCBI database.
- Antibacterial activity of synthesized MONPs against isolated gram positive and negative bacteria.
- Antifungal activity of synthesized MONPs against isolated fungi.

5.2.3 Observations to be recorded: The results will be obtained by measuring radial inhibition zones in bacteria and percent inhibition (%) in mycelial growth of fungi. The synthesized nanoparticles which overall show best antimicrobial activity will be further carried for third objective.

5.2.4 Statistical analysis: Completely randomized block design, 2 factor analyses will be used.

5.3 Experiment 3: Storage study of perishable vegetables with metal oxide nanoparticles.

5.3.1 Location: Eternal University

5.3.2 Methodology:

- Crops variety and location

Crop	Variety	Location
Capsicum	Indra	Machher
Tomato	Solan Lalima	Machher

- Dip coating method by using different concentrations of MONPs with biodegradable polymer is used to evaluate the storage study of 1-20 days at ambient temperature (27ºC) on capsicum and tomato obtained from local farmers.

5.3.3 Observations to be recorded: Physical analysis; weight loss, length, and width, Proximate analysis; moisture, fat, fibre, ash content,

Biochemical analysis; chlorophyll content and ascorbic acid test, lycopene test will be studied.

5.3.4 Statistical analysis: Mean differences of all the parameters, at different storage times at ambient temperature will be compared by using complete-randomised block design.

5.4 Experiment 4: Toxicity evaluation of synthesized metal oxide nanoparticles against HEK293 cell line.

5.4.1 Location: Eternal University or IIT Delhi

5.4.2 Methodology:

- Toxicity evaluation against normal Human embryonic kidney cell line (HEK293).
- Cell lines grown in Dulbecco's Modified Eagle Media (DMEM) at 37 ºC and 5% CO_2 concentration.
- Diversify treatment of MONPs at different doses (10-1000µg/ml) will be given at certain temperature.

5.4.3 Observations to be recorded: Cell viability will be expressed as a percentage relative to the untreated control cells. Cell viability % =OD/CA*100, where OD is the optical density whereas CA is control average.

5.4.4 Statistical analysis: Nil

5.4.5 Plan of Research Work-

Time schedule of activities and activity milestone

Sr No. .	**Objectives**	When to be attained					
		1st Year		2nd Year		3rd Year	
		I	II	III	IV	V	VI
1.	Synthesis and characterisation of metal oxide nanoparticles using hydrothermal method.						
2.	Antimycotic and antibacterial study of synthesized metal oxide nanoparticles against opportunistic pathogenic fungi and bacteria.						
3.	Storage study of perishable vegetables with metal oxide nanoparticles.						
4.	Toxicity evaluation of synthesized metal oxide nanoparticles against HEK293 cell line						

6. Collaborations:

1) Dietetics and nutrition technology division, CSIR IHBT, Palampur (H.P.)
2) Centre for Biomedical Engineering, IIT, Delhi.

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Author Name	Geetika Guleria (BS19PSBT004)
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Department	Genetics, Plant Breeding and Biotechnology
Acceptable Maximum Limit	10%
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Paper Title	SYNTHESIS, CHARACTERIZATION, ANTIMICROBIAL & TOXICOLOGICAL EFFECT OF METAL OXIDES NANOPARTICLES
Similarity	8%
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Signature of Major Advisor

17/12/2022

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APPENDIX-I

ADVISORY COMMITTEE

Name	Designation	Department	Signature	
Dr. Sapna Thakur	Assistant Professor	Genetics, Plant Breedy and Biotechnology	Sapna	Major Advisor
Dr. Roop Singh Bard	Associate Professor	Genetics Plant Breedy and Biotechnology	Roop Singh	Member
Dr. Sushma Sharma	Assistan Professor	Plant Pathology	Sushma	Member
Dr. Poonam Kumari	Assistant Professor	Physics	Poonam	Member
Dr. Kamal Kishor	Associate Professor	Chemistry	Kamal	Member

Forwarded five copies to the Dean, Postgraduate Studies for approval by the RDC

18/02/20

Head of the Department/ Principal

18-2-2020

Dean of the College

Dean Post Graduate Studies

Eternal University
Baru Sahib (HP)
Report of the Research Degree Committee on Synopsis of Master's /Ph.D Thesis/Dissertation Research Problem

We have critically examined the synopsis of Thesis/Dissertation Problem entitled : **SYNTHESIS, CHARACTERIZATION, ANTIMICROBIAL AND TOXICOLOGICAL EFFECT OF METAL OXIDES NANOPARTICLES** submitted by : **Geetika Guleria** Registration No : **BS19PSBT004** for M.Sc/M. Tech/Ph.D degree in the subject of: **Biotechnology** to the Dr. KS Gill Akal College of Agriculture of the Eternal University, Baru Sahib(HP). It is certified that the research problem in original; programme of work is properly planned, feasible to carry out and is of good academic and practical significance.

Ms/Mr.: **Geetika Guleria** gave a presentation on the above mentioned research problem to the Research Degree Committee and other faculty members and students on **March 09, 2022** and his/her performance is Satisfactory/~~Unsatisfactory~~.

Synopsis is recommended for acceptance in by the Eternal University.

09.3.2022

(Signature, Name and Address of External Expert)

(Dr. Manisha Thakur)
Associate Professor
Department of Biotechnology
Dr. Y. S Parmar, UHF, Nauni, Solan

1. _____
(Chairperson, Research Degree Committee)
(Lt. B.S. Sohal, Dean PGS)

2. _____
(Member, Research Degree Committee)
(Dr. SK Sharma, Dean DKSGAC)

3. _____ 9/3/2022
(Member, Research Degree Committee)
(Dr. Sapna Thakur, Major Advisor)

4. _____
(Member Research Degree Committee)
(Dr. Roop Singh Bora)

5. _____ 9/3/22
(Member, Research Degree Committee)
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6. _____ 9/3/2022
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(Dr. Poonam Kumari)

Recommendation

(Dean, Post-graduate Studies)

Approved/Not Approved

Vice Chancellor